A NOTABLE SOURCE OF ERROR IN TESTING GASEOUS DISINFECTANTS.

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THE testing of gaseous disinfectants for public health purposes is done for two general objects, in two different ways. The first object is that sought when the investigator determines from a large number of experimental tests the required amounts of gas and best methods of work, and then prescribes these for use in practice. The second object is to secure a check system by which each particular disinfection performed in practice is tested as a routine procedure by the exposure in the room of one or more test organisms, the death of which is required before the disinfection is officially approved. The two systems supplement each other, but the second is scarcely required if the disinfection is performed strictly according to a proper prescription, and is intended primarily as a test of the disinfector rather than of the disinfectant. For whatever purpose the tests are made, it is rather obvious that they should be so designed that the survival of the test organism should be good evidence that the room treated would have remained infected, had it been originally infected naturally by a patient suffering from an infectious disease, and also—*vice versa*—that the death of the test organism should prove that the room treated would no longer be infective, if it were infective before disinfection was done. It is of the greatest moment, therefore, that the condition of the test organisms, as well as their character, should parallel closely, or, if possible, be identical with, the condition of the infective agents which are, or are supposed to be, present in rooms occupied by infected patients.

The writer has had occasion to point out in previous articles[1] the probable position and condition of infective agents in naturally infected rooms. It is now his object to point out, from accumulated evidence, the requisites which should be demanded of test organisms

[1] *Am. Pub. Health Assoc. Rep.*, 1902, 28, pp. 209, 509; *Bulletin*, Vermont State Board of Health presented at the Vermont summer school for health officers, 1903.

used in testing disinfectants, these requisites being based on the premise above given, i.e., that the test organism should parallel in condition, as well as character, the infective agents which it is desired to destroy.

It has been pointed out by a number of investigators that the efficiency of gaseous disinfectants, chlorine, sulphur, formaldehyde, carbolic acid vapor, etc., is dependent very largely on the humidity of the atmosphere in which they act. In saturated atmospheres remarkably small amounts of these gases are efficient. In dry atmospheres, they are practically inert, even if present in relatively large quantities. But a most important point is this, that a dry gas, acting upon a moist organism, kills it just as surely as does a moist gas acting upon a dry organism. This very simple fact has led to much of the confusion of results in work carried on by different observers, who, attempting to obtain similar results by similar methods, obtained contradictory results because of the differences in the degree of moistness of the test organisms respectively used; while another set of contradictions has resulted from differences in the humidities of the atmosphere in different tests. With two such important variables almost entirely overlooked in most "practical" disinfection tests of gaseous disinfectants, it is not astonishing that one observer records excellent results from the use of a method which, in the hands of another, utterly fails.

The work upon which this paper is based was done in the attempt to reconcile two sets of absolutely contradictory results obtained, one set in Boston, the other in the hands of a high authority not far away from Boston. A third, and more unusual, source of error was incidentally discovered—the drying of test organisms to an unusual extent; the discovery resulting finally in a statement from one observer that the sum total of favorable results he had so far obtained was absolutely worthless, since his controls themselves did not survive the period of drying employed, without the use of any disinfectant at all.

Briefly summed up, the facts are that test organisms, fresh and moist, are very susceptible to small quantities of disinfectant gas, dry or moist; the same organisms, fresh but dried, are extremely resistant to the same amounts of dry gases; while, finally, the same organisms, dried

for a week or more, lose vitality to such an extent that even small amounts of gas, not necessarily at high humidities, will kill them. The writer has exposed in the same room, under identical conditions and at the same time, the same organisms on filter paper, and kept all night, one-half in such a manner as to dry thoroughly, the other half in such a manner as to remain moist. The moist organisms were all killed, the dry organisms all survived. Test objects prepared so that an intermediate stage of dryness was reached, behaved irregularly, some surviving, some perishing. Moreover, organisms (B. pyocyaneus) prepared on successive days, in such a manner that at the end of a week, all could be exposed under identical conditions to the same gas, gave results which showed that those prepared immediately before the exposure (i. e., still moist) were killed, those prepared two, three, and four days before (i. e., well dried, but still fresh) survived; while those prepared five, six, and seven days before were killed in numbers proportionate roughly to the age of the specimen, those longest dried showing the largest proportion of killed.

The writer's object in submitting this statement at this time is to point out certain sources of error which have in the past given to those concerned in selecting methods of disinfection a bewildering set of contradictory data to digest. Some of those to whom such contradictory results have been submitted have naturally enough become disgusted with all results of the same nature. Nor is it to be wondered at, when, for instance, one city adopts, on bacteriological evidence, five ounces of formaldehyde per 1,000 cubic feet, and another city, also on bacteriological evidence, has announced officially that over 70 ounces per 1,000 cubic feet is not uniformly efficient. The question will naturally arise: What character and conditions should a test organism have to yield a satisfactory and conclusive result? The answer is involved in what has been said already. Categorically, it should be fresh—not more than two days dried; and it should be really dry; while the species used should be those encountered in actual practice, or non-pathogenic forms, carefully tested and selected to show parallel resistance to those which it is desired to kill in practice. The reason for selecting dry organisms, dried, however, not more than two days, is simply that such organisms represent the conditions which those infective agents naturally distributed in the

infected room will most probably present. Any organisms in the room freshly deposited by the patient just before death or removal, are likely to be moist, hence are likely to be killed readily. Those deposited by the patient a week or more before are likely to be dead, or at least dried to a point of low resistance, and are also readily killed. It is therefore the organisms that were thrown out by the patient from one to seven days before his death or removal that will usually prove the more resistant, and it is to these that the disinfector must pay attention. It is obvious that organisms of like age and dryness should be used for the tests. To kill organisms in this condition high humidity of the atmosphere is required if efficiency, with economy of gas, is desired.

In practice, bacteria dry more quickly on glass than on cotton or filter paper. This is probably the chief reason, if not the only one, why it has been noted by various observers that organisms dried on glass are more resistant (if not dried too long) than those dried on the other materials under like conditions. Hence glass objects for test organisms have, in the writer's hands, given the most generally reliable and uniform results.